論文の内容の要旨

Development of a Morphological Indicator of Microalgal Cu^{2+} Removal Efficiency

（微細藻類の Cu^{2+}除去効率の形態インジケーターの開発）

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Heavy metal removal from wastewater is one of the most concerned worldwide environmental problems because heavy metals are stable and hazardous elements, which are non-biodegradable and could be bio-accumulated. To effectively eradicate toxic heavy metals from wastewater and stabilize the ecosystem, various methods have been reported, which fall into two categories: conventional physico-chemical methods and bioremediation methods. Among them, microalgae-based bioremediation methods have attracted much attention in the past few decades for treating wastewater polluted with heavy metal because of the increasing demands of more environment friendly, cost-effective, and sustainable treatment methods. However, the heavy metal removal efficiency of microalgae is far from practical use. A small amount of research has investigated the improvement of heavy metal removal efficiency of microalgae, such as immobilization, pretreatment, and genetic modification of microalgal cells. But these methods either have limited enhancement of the HM removal efficiency of microalgae or

potentially pose threat to the environment and human health by deploying genetically modified species.

To address these problems, directed evolution is expected to be effective for developing microalgae with much higher HM removal efficiency without the problem of using new strains in wastewater treatment. However, experimental demonstration of this method is challenging because there is no non-invasive or label-free indicator to identify the highly efficient cells. The presently available indicators, like the ion intensity of heavy metals within each cell and the fluorescence intensity of metal fluorescent probes, are invasive or require labelling.

In this doctoral work, I proposed and experimentally demonstrated an intelligent cellular morphological indicator for identifying the heavy metal removal efficiency of *Euglena gracilis* in a non-invasive and label-free manner. Specifically, I recognized a morphological meta-feature of *E. gracilis* cells under 7.5 μM Cu^{2+} exposure by analysing single-cell images via machine learning algorithms, and demonstrated a strong monotonic correlation (Spearman's $\rho = -0.82$, $P = 2.1 \times 10^{-5}$) between this morphological meta-feature and the Cu^{2+} removal efficiency of nineteen *E. gracilis* clones. Our findings firmly suggest that the morphology of *E. gracilis* cells can serve as an effective HM removal efficiency indicator. The intelligent morphological indicator has great potential, when combined with the recently developed powerful sorting methods such as the high-throughput image-activated cell sorter, for directed-evolution-based development of *E. gracilis* with extremely high HM removal efficiency for practical wastewater treatment worldwide.